THE EFFECT OF SPERMIDINE ON THE RATE OF SEX-LINKED RECESSIVE LETHALS CAUSED BY X-RAY IN *DROSOPHILA MELANOGASTER*

A Thesis

Presented to

The School of Graduate Studies

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

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May 1984

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An abstract of a Thesis by
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The problem. Spermidine is a polyamine naturally occurring in many organisms including *Drosophila melanogaster*. It has been shown to decrease the heat denaturation of DNA by binding to the DNA double helix. The agent has also decreased the development of resistance of bacteria to a particular antibiotic. The major objective of this study was to determine if treatment to *Drosophila* with a given concentration of spermidine prior to X-ray exposure would change the expected number of sex-linked recessive lethal mutations.

Procedure. Adult male wild-type *Drosophila* were treated in four ways. The four treatments, administered via feeding in a medium with dimethyl sulfoxide, were Control, X-ray, spermidine, or spermidine and X-ray. Treated adult males were mated in groups of twenty with twenty virgin *Basc* females. The *Basc* technique for detection of X-linked recessive lethal mutations was followed. Mutation frequencies were calculated and data was submitted to the Kastenbaum-Bowman test.

Findings. The control group exhibited a mutation frequency of 0.68% which showed no significant difference compared with the 0.59% mutation frequency with spermidine. The mutation frequency for the X-ray only treatment was 2.62% and the mutation frequency for the spermidine and X-ray treatment was 2.86%.

Conclusions. The data obtained from this study showed that pretreatment with spermidine had no significant effect on decreasing the expected number of sex-linked recessive lethal mutations caused by X-irradiation exposure. Spermidine alone was shown to have no mutagenic effect, but in combination with irradiation a slight, statistically insignificant, increase was found.

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INTRODUCTION AND REVIEW OF THE LITERATURE

Mutation studies are an important part of genetic research, because spontaneous mutations are related to evolutionary changes and induced mutations may result from the contacts humans or domestic animals have with environmental mutagens. The rate at which induced mutations occur does not only depend on the dose and agent used as the mutagen, but also on any external or internal factors acting on the organism. Though these factors are numerous, introducing and studying a single factor may provide a better understanding of how the mutagen works, or it may give information on a potential antimutagenic agent. Substances present during mutagen exposure that are known to interact physically with DNA are especially likely to depress mutagenic activity. As a result, these substances are a prime area to study. One class of substances which exist naturally in organisms and interact with DNA is polyamines. The present study investigated the potential antimutagenic properties of one polyamine, spermidine. The study tested the hypothesis that spermidine is an antimutagenic chemical and can decrease the effects of a mutagenic agent.

Polyamines are nonprotein nitrogenous bases that have been shown to have biological significance in a variety of ways. Polyamines, in particular spermidine (H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2) and spermine (H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-

NH_2) were found in several animal tissues as early as 1925 (Dudley and Rosenheim 1925). Rosenthal and Tabor (1956) analyzed the content of spermidine and spermine in various organs and found human semen, ox liver, kidney and spleen to contain relatively large amounts of spermine. Polyamines have also been found in the nervous system of several mammals including rabbits, mice, and cats (Bachrach 1970).

One of the most striking and studied aspects of the polyamines, and one which is particularly relevant to the present study, is their ability to bind to nucleic acids. When tritiated spermine was incorporated into spheroplasts of E. coli, 60-70% of the spermine was contained in a sedimentation fraction of DNA and RNA (Johnson and Bach 1968). The binding between polyamines and nucleic acids involves an electrostatic noncovalent attraction linkage between the basic, positively charged amine group of the polyamines and the highly acidic, negatively charged phosphate group of the nucleic acid (Bachrach 1970). The complexing results in a neutralization of the negatively charged phosphate groups that usually repel each other, so that the nucleic acid is condensed (Tabor and Tabor 1964). Liquori and coworkers (1967) demonstrated via an X-ray analysis of crystal structures that the polyamines have a shape and dimension which allows the molecules to undergo a stereospecific interaction with DNA; the polyamine lies in the narrow grooves of the macromolecule. Within the

narrow grooves the polyamines form hydrogen bonds with the oxygen molecules of the phosphate groups of DNA (Liquori et al. 1967).

The ability of spermidine to increase the stability of the double helix of the DNA molecule has been found to have several effects. Flink and Pettijohn (1975) showed that DNA isolated from E. coli showed a decrease in unfolding, as measured by sedimentation rate when stored in the presence of spermidine at concentrations of 5 or 10 mM. Tabor (1962) found that the temperature required for denaturation of calf thymus DNA increased substantially with the addition of various polyamines. Tabor proposed that the most likely explanation of the stabilization was the effective neutralization of the phosphate groups of the DNA by complex formation with the amines. This consequently increases the effective strength of van der Waal's forces and hydrogen-bonding forces, leading to increased stabilization.

This stabilization by polyamines has also been found to have certain antimutagenic properties. In bacterial mutation research, spermine in combination with an antibiotic prevented the emergence of drug-resistant strains from Staphylococcus aureus, Escherichia coli, and Aerobacter aerogenes (Sevag and Ashton 1964). Spermine was found to cause an even more striking effect in decreasing the mutation rate when the mutations were induced by inclusion of caffeine in the culture medium or exposure to

ultraviolet light (Johnson and Bach 1965). Mutagenesis by ultraviolet light is believed to be caused by the formation of pyrimidine dimers or cross-links in the DNA which subsequently will cause misreading during replication of the DNA. Perhaps the polyamines counteract this process by promoting repair; more likely they minimize the likelihood of misreading a strand which has uncorrected errors in it (Bach and Johnson 1971).

Although several lines of investigation have given definite information on polyamine-nucleic acid interactions in vitro, the role of polyamines in vivo is still unclear. Tabor and Tabor (1964) pointed out two primary problems with isolating and determining the role of natural polyamines: (1) it is difficult to localize the amines within the cell and knowing in particular where they are present in high concentrations within the nucleus; (2) compounds such as histones, protamines and polylysine also precipitate nucleic acids and protect DNA from heat denaturation so it may be difficult to isolate spermidine as the active agent. In a review by Tabor and Tabor in 1976, the in vivo relationship between polyamines and nucleic acids was still unclear. Even gross effects of administration of polyamines to an animal were virtually unknown until Shaw (1972) determined that animals became sedated, hypothermic and hypomotile if they received intraperitoneal injections of polyamines. However, polyamines have been found to

naturally be present in Drosophila melanogaster. Herbst and Dion (1970) showed that spermidine is the predominant polyamine present, but the specific tissues were not mentioned. They determined that the highest concentration is found during early larval stages with minimum amounts occurring in the prepupal stage. There is an increase in spermidine to intermediate levels during histogenesis of pupae and low intermediate levels are maintained in the adult (Herbst and Dion 1970). These changes in spermidine levels in Drosophila were again supported in findings by Byus and Herbst (1976) in which they found that decarboxylase activities, agents which catalyze an essential step in production of spermidine, was highest during early development and lowest in older larvae.

Though spermidine has been found naturally in Drosophila, in order for exogenously introduced spermidine to have the potential to act as an anti-mutagenic agent it must reach the DNA of the cells. The method of spermidine introduction into the organism is one aspect to be considered. Herbst and coworkers (1973), in an experiment on the effect of spermidine on RNA synthesis, chose to administer the polyamine via feeding to the larval stage of Drosophila. Though spermidine was shown to have the ability to penetrate larvae cell membranes (Herbst et al. 1973), a preliminary project showed a potential problem when using larvae for the present study. It would be very

difficult to keep the larvae in contact with the spermidine for a twenty-four hour period because they would crawl off the absorbant toweling onto the lid of the petri dish. Thus different larvae might receive very different doses. To avoid this problem, spermidine pretreatment for the present study was done by feeding the adults, since adults are known to feed continuously.

The other factor to consider in relation to spermidine's ability to reach the DNA of the cells is the metabolism of the spermidine. Siimes (1967) showed that spermine and spermidine are oxidized to carbon dioxide in vivo in mammals. The polyamines are synthesized in developing rats and maintained at carefully controlled levels in particular tissues regardless of diet (Rosenthal and Tabor 1956). However administered, spermine concentrated in the kidney, and a small portion (4-21%) of the administered dose appeared in the urine at 24 hours (Rosenthal and Tabor 1956). Approximately equal amounts of spermidine were also found; it arose as a degradation product.

Though the metabolism of polyamines is still an active research area today, the research seems to be directed towards specific actions in vertebrates, and therefore no further metabolic information will be introduced here because of a lack of relevance to the present study on the possible anti-mutagenic effects of polyamines in Drosophila. At this time, in vivo work on invertebrates

and polyamines is very limited.

In order to test the potential antimutagenic action of spermidine, a particular mutagen must be selected. One of the best studied mutagens and the one used in this study is X-irradiation. The first definite proof of its mutagenic action was obtained by Muller (1927, cited by Auerbach 1976) when he worked with the prime organism of genetic studies, Drosophila melanogaster. From this early work by Muller, it was determined that X-rays cause a significant increase in the number of sex-linked recessive lethal mutations. These mutations are initiated by the contact of the cell or chromosome with highly reactive ionizing photons (Wallace and Dobzhansky 1959). The photons only persist briefly in the cell; if contact is made with the chromosome, it can interact with the genetic material to induce damage at the chromosome level or the gene level (Wallace and Dobzhansky 1959). The damage may be in the form of a chromosome break which commonly results in reattachment as in a translocation, insertion or inversion, or no reattachment as in a deletion (Muller 1954). Damage may also be at the gene level producing a point mutation. This was the type considered in the present study.

Though at one time these point mutations and chromosome alterations were believed to be due to a single "hit" of radiation (Lea 1955), this theory is less popular today, and other actions of X-ray on the nucleic acids have since

been proposed. Due to the nature of the pretreatment utilized in this experiment and the fact that it was predicted that the spermidine would stabilize the DNA helix, it is important to consider the effects of X-irradiation that might be influenced by helix stability. Since X-irradiated solutions of DNA show a decrease in viscosity, two possible mechanisms have been proposed for how the X-irradiation affects the DNA (Arena 1971). The first mechanism deals with the fact that normally the DNA helix is stiff and therefore it cannot fold or become more compact. X-irradiation causes a decrease in the stiffness so the helix may become more compact. Since the polyamines have been shown to increase the stabilization of DNA, they may act to inhibit folding and keep the DNA in its characteristically stiff conformation. Alternatively, a decrease in viscosity may also result if molecular degradation occurs by both strands of the helix breaking less than five nucleotides apart so the helix is fragmented. The neutralization caused by spermidine may also act to inhibit this action of X-irradiation.

After X-irradiation was chosen as the mutagen for the present study, the dose of irradiation was considered. Since only one specific dose was to be used, it was important to choose one which had a noticeable effect, but not one which would cause extensive lethality due to immediate damage to somatic cells. Shortly after Muller's experiments

in 1927, interest grew in the relationship between dose of radiation and the number of sex-linked recessive lethal mutations. This interest has resulted in a large amount of research in this area. According to Muller (1962), in the early decades, 1928-1948, following his original work in 1927, it was demonstrated that the relationship of frequency of sex-linked recessive lethal mutations to dose is substantially linear over a very wide range. If the effect of a mutagenic agent is linear, the expected mutation rate can be conveniently related to the number of organisms necessary to detect a change in rate, and need not take threshold effects into account. Spencer and Stern (1948) showed that linearity held for doses of X-ray as low as 25 R, but it is unlikely linearity will hold to a dose of zero. At doses lower than 3000 R, more than two-thirds of the recessive lethals are associated with a decrease in dose. This is interpreted to mean the linearity principle for recessive lethal mutations applies primarily to point mutations, since chromosomal aberrations involve a cytologically visible structural change (Muller 1962). At higher doses, above 2000-3000 R, departure of linearity may occur because there may be two or more lethals induced in the same chromosome and this would only be scored as a single lethal (Wallace and Dobzhansky 1959). As a result of this information on linearity, a dose of 2000 R was chosen for the present experiment.

Though there is a variety of techniques to test the mutagenic effect of X-rays, one of the most commonly employed is the test for sex-linked recessive lethal mutations. The test was first used by Muller in 1927 in his early mutation studies. The test is designed for Drosophila, and they are particularly convenient to use because of a short generation time of ten days and the low cost of the culture media (Lee et al. 1983). Two stocks are commonly employed for the study. The wild type is used as the "tester" strain, the strain which receives the treatment under study. The Basc stock is used as the "balancer" strain to be mated with the X-irradiated flies. The Basc female carries on her X-chromosome a system of two overlapping inversions and two marker genes, apricot eye color (w^a) and Bar eye shape (B) (Auerbach 1976). The marker gene allows the researcher to distinguish a F_2 male with the Basc X-chromosome from males with the wild-type chromosome which was treated in the P_1 generation. The overlapping inversions prevent crossing over, which would confuse matters.

In the Basc mating scheme, each F_1 female represents and carries one treated wild-type X-chromosome. If the treated X-chromosome has sustained a lethal mutation, all F_2 males receiving it will not appear. Only Basc males with the lethal-free X-chromosome may survive.

Though the test for sex-linked recessive lethal mutations can be used to study the effects of a single

mutagen, it will detect any recessive sex-linked lethal mutations, so it is ideal for research on potential mutagen enhancers or inhibitors. An early study of substances inhibiting mutagenesis was the work done with the antibiotics penicillin (Shiomi 1966; Burdette 1961a) and Actinomycin D (Burdette 1961b). In the studies of Shiomi and Burdette, when adult flies were fed the antibiotic, a decrease occurred in the sex-linked recessive lethal mutation rate following exposure to X-ray. Clark (1963, cited by Shiomi 1966) injected penicillin into Drosophila and found no decrease in mutation frequency. As a result, Shiomi (1966) proposed that the antibiotic had a secondary effect having some relation to the metabolic activity of the developing germ cells.

Though it is unlikely penicillin acts at the DNA level, actinomycin binds to DNA and has the ability to inhibit RNA synthesis (Cohen and Yielding 1965). Both antibiotics did inhibit the expected number of sex-linked recessive lethal mutations caused by X-ray; therefore these antimutagenic studies were chosen as procedural models for the present study. Specifically, their feeding technique of administration was used. In order to enhance the ability of the spermidine to enter the generally impermeable organism, dimethyl sulfoxide (DMSO) was used in the present study. DMSO is frequently used in genetic mutation studies because of its ability to enhance the permeability of cell

membranes, and its non-toxic qualities (Kopeck 1973; LeRoy 1972; Aschenbrener 1972; Alexander 1966, cited by Kopeck 1973). Kopeck (1973) used DMSO to carry the mutagen ethyl methanesulfonate (EMS) into Drosophila, via a topical administration, to test the anti-mutagenic properties of DMSO. DMSO was found to have no significant effect in decreasing the number of sex-linked recessive lethal mutations expected by EMS, in fact 100% DMSO alone was found to enhance the spontaneous mutation rate. DMSO alone at lower concentrations was found to show no significant increase in the number of X-linked recessive lethal mutations (LeRoy 1972; Aschenbrener 1972).

The present experiment was designed to test the effect of a specific concentration of spermidine in inhibiting the expected mutation rate caused by a given radiation dose. Spermidine was chosen due to its ability to stabilize DNA. X-irradiation was chosen as the mutagen because it is the most convenient and potent mutagenic agent available. DMSO was used to enhance the passage of spermidine into the Drosophila.

METHODS AND MATERIALS

Oregon-R (OR) and Basc strains of Drosophila melanogaster were used in this study. Oregon-R, a wild-type strain, received the treatments. The Basc strain was chosen because the X-chromosomes of this strain carry two overlapping

inversions and two marker genes, apricot eye color (w^a) and Bar eye shape (B).

P_1 matings were made in quarter-pint glass bottles with 35 ml of cornmeal, agar, and molasses medium. The F_1 matings were made in 25 x 105 mm shell vials containing 6 ml of Carpenter's medium-agar, sucrose, brewer's yeast and salts (Carpenter 1950). All crosses were maintained in constant light at a temperature of 23° ± 2° C.

Spermidine (Sigma) was tested in the study for its possible interaction with ionizing radiation. Dimethyl sulfoxide (Fisher Scientific - ACS Reagent Grade) was used, as a standard part of all four treatments, to increase the permeability of the cell membrane. The X-rays were administered with a Picker Zephyr unit, through the courtesy of the Physics Department at Drake University.

Oregon-R males, 72-96 hours old, were treated in one of the following ways: (1) Control-DMSO only, (2) DMSO and X-ray, (3) DMSO and spermidine or (4) DMSO, spermidine and X-ray. All of the treated males were fed in quarter-pint bottles from a piece of absorbant paper containing solutions for a specific treatment for a twenty-four hour period. They were then mated with virgin Basc females immediately or following irradiation depending on the group.

The control group was fed on 1 M DMSO in Carpenter's media lacking brewer's yeast and agar.

Males constituting the X-ray group were allowed to

feed on 1 M DMSO, as the controls did, and after the twenty-four hour period the flies were irradiated using 64 kilovolts of 5 ma, 6 1/8 inches from an unfiltered X-ray source for 4 minutes 39 seconds giving a dosage of 2000 R.

The spermidine group was fed on 100 mM spermidine which was made by diluting the spermidine with the 1 M DMSO.

The spermidine and X-ray group, following feeding on the 100 mM spermidine, was irradiated along with the X-ray only group using an identical schedule of irradiation.

In all four groups, ten quarter-pint bottles containing twenty treated OR males and twenty virgin females were set up. After two days on the medium, halfway through the expected egg laying period, the flies in each of the bottles were transferred to a new bottle of medium to prevent overcrowding of the progeny. At the end of the two day period on the second bottles, all adult flies were discarded.

F_1 x F_1 matings were made using one female of the F_1 progeny and two males. If there was a shortage of F_1 sibling males, males from Basc stock cultures were substituted since the F_1 males have the same X-chromosome as a Basc stock culture male.

All of the crosses described above were performed twice.

Any such F_2 cultures containing a lethal mutation will have only three instead of four classes of progeny present. Half of the female progeny will be heterozygous

Basc females and the other half will be homozygous Basc females. The male progeny will only be made up of Basc males. There will be an absence of the wild-type males if the particular F_1 female used for this culture carried a lethal on the treated wild-type X-chromosome which would have been transmitted to this class of sons. Any F_2 culture which exhibited the absence of the wild-type male and contained at least seven Basc males was scored as containing an X-linked recessive lethal mutation.

Vials containing fewer than seven Basc males and no wild-type males were scored as probable lethals. Since the probability of producing six Basc males and no wild-type males just by chance is 1/64, it is believable, even though improbable, that the wild-type males could be absent from a small group of males in an F_2 culture in which there was really no lethal mutation. When fewer than seven Basc males were recovered from a culture lacking wild-type males, a few of the heterozygous Basc F_2 females were mated individually to two Basc males. Absence of wild-type male progeny in the F_3 generation in all sub-matings confirmed the presence of a lethal mutation beyond a reasonable doubt.

All results were submitted to the Kastenbaum-Bowman test (1970) because the total number of mutations was less than 100.

RESULTS

The represented results from both trials are presented in Table 1. It is evident from the values obtained with the Kastenbaum-Bowman test (1970) that the mutation frequencies between the two trials had no highly significant difference for any of the treatments using the 0.01 confidence level. At the 0.05 confidence level the spermidine-only treatment showed a significant difference in mutation frequencies between the two trials. Despite this difference, the data from both experimental trials was combined, for further study, in Table 2 since there was no evidence of any large differences in mutation rates. All evaluations will be based on this table. All comparisons that follow between treatment groups are based on calculations using the Kastenbaum-Bowman test (1970) at the 0.05 confidence level.

The group of Drosophila treated with 100 mM spermidine in the 1 M DMSO for twenty-four hours had a mutation frequency of 0.59% (Table 2). When this rate is compared with the mutation frequency of the control group, no significant difference in the mutation frequencies is observed between these two groups.

The Drosophila treated with X-ray only had an observed mutation frequency of 2.62% (Table 2). Comparing the X-ray only group with the control group, the X-linked recessive lethal mutation frequencies are shown to be

Table 1. Results of the Basc, X-linked recessive lethal mutation tests for each experimental trial

Treatment	Experimental Trial	Total F_1 Matings	Fertile F_1 Matings	Non-Lethal F_2 Tests	Lethal F_2 Tests	% Lethal Tests
Control	1	548	536	532	4	0.75
	2	654	642	638	4	0.62
Spermidine	1	683	661	660	1	0.15*
	2	717	706	699	7	0.99*
X-ray	1	497	485	469	16	3.30
	2	638	620	607	13	2.10
Spermidine & X-ray	1	676	658	640	18	2.74
	2	724	707	686	21	2.97

*Significant difference at the 0.05 level between trial 1 and 2.

Table 2. Results of the combined Basc, X-linked recessive lethal mutation tests

Treatment	Total F_1 Matings	Fertile F_1 Matings	% Fertile F_1 Matings	Non-Lethal F_2 Tests	Lethal F_2 Tests	% Lethal Tests
Control	1202	1178	98.0	1170	8	0.68
Spermidine	1400	1367	97.6	1359	8	0.59
X-ray	1135	1105	97.4	1076	29	2.62*
Spermidine & X-ray	1400	1365	97.5	1326	39	2.86*

*Significantly different from the control group at the 0.05 confidence level.

significantly different.

The mutation frequency of 2.86% (Table 2) obtained for the group of adult OR flies fed on the 100 mM spermidine diluted in 1 M DMSO and then irradiated with 2000 R X-irradiation was compared with the control group. The value obtained indicates that the number of mutations observed in the spermidine and X-ray group and the control group is significantly different.

The last statistical comparison was made between the X-ray only group and the spermidine and X-ray group. More mutations were obtained with spermidine than without it, but the difference was not statistically significant. The estimation of the confidence limits of a proportion (Snedecor and Cochran 1967) was also calculated for these two groups, and it is indicated from these values, 1.7% to 3.5% for X-ray and 2.0% to 3.8% for spermidine and X-ray, that at the 95% confidence level spermidine does not double or halve the mutation rate. Both of these statistical tests indicate there is no significant difference in the number of X-linked recessive lethal mutations observed between these two groups.

The fertility rates for all of the F_1 matings for all four treatments were between 97.4% and 98.0%.

DISCUSSION

The present study was an attempt to determine if a specific concentration of spermidine could change the number of sex-linked recessive lethal mutations caused by X-irradiation. When the X-ray treatment mutation frequency of 2.62% is compared with the spermidine and X-ray mutation frequency of 2.86%, there is no significant difference in mutation rates. As a result, several conclusions may be drawn. The first one is that the data refutes the hypothesis that the tested dose of spermidine reduces the X-linked recessive lethal mutation rate caused by X-irradiation. This hypothesis was proposed due to the previous work with spermidine's ability to bind to DNA and its antimutagenic properties (Johnson and Bach 1968; Bachrach 1970; Tabor and Tabor 1964; Liquori et al. 1967). Since irradiation causes point mutations, the spermidine might bind to the DNA double helix and prevent some of the mutations otherwise caused by X-irradiation.

Spermidine did not decrease the number of sex-linked recessive lethal mutations recovered. In fact, there were a few more mutations with spermidine. Several explanations may be offered as alternatives to refuting the hypothesis. First, it is possible that the spermidine never reached the germ cells of the adult Oregon-R males. It had been supposed that the DMSO would make cells more permeable to

spermidine since DMSO penetrates the intact dermis (Herschler and Jacob 1965, cited by Kopeck 1973) and mucous membranes (Jacob et al. 1964, cited by Kopeck 1973). David (1972, cited by Kopeck 1973) proposed that the mechanism for the ability of DMSO to penetrate skin and mucous membranes may have been the result of its dynamic interaction with tissue water by forming hydrophobic bonds between DMSO and exposed moieties (N-H and O-H) of the protein structure. Kopeck (1973) found DMSO was effective in transporting EMS into Drosophila. Future studies might use radioactive spermidine to determine whether DMSO really does carry spermidine across cell membranes. It does seem likely that some spermidine passed into the Drosophila, because the flies were on the absorbant paper with the 100 mM spermidine for twenty-four hours. Drosophila dehydrate in that time span if no liquids are taken in.

If it is assumed that the spermidine was taken in by the Drosophila the next problem concerns the metabolism of spermidine. Dion (1970) demonstrated that the administration of [^{14}C] spermidine to larvae older than 104 hours resulted in the rapid production of labeled putrescine, which was rapidly metabolized. His studies also showed that pupal stages maintained at 0° C exhibited rapidly reduced levels of spermidine probably as a result of acetylation. Dion (1970) concluded through his research that stringent homeostatic control exists over spermidine levels. In the

present study, the spermidine introduced may have been rapidly converted to putrescine and then further metabolized. This rapid metabolism might prevent the spermidine from reaching the germ cells and binding to the DNA.

Another possible reason for doubting the obvious conclusions of the experiment is that the concentration used may have been too low to have an effect. The spermidine concentration used, 100 mM, was chosen after looking at work by Herbst et al. (1973) with Drosophila larvae. They used 1 mM and 10 mM concentrations of spermidine in a liquid culture medium in which the larvae were incubated for four hours. Herbst et al. (1973) was unsuccessful in determining the toxicity level, but the highest concentration used, 0.12 M, did not affect the larvae as judged by normal movement in the media. In choosing the 100 mM concentration for the present study, an attempt was made to use a concentration as high as possible without causing detrimental effects to the Drosophila. Since this concentration, 100 mM, had no effect, finding the LD50 level for spermidine could be beneficial for future studies. The sex-linked recessive lethal test could be used with an LD50 concentration in conjunction with X-irradiation. The LD50 concentration of spermidine might in turn increase the level of spermidine in the Drosophila germ cells causing a more pronounced phenotypic effect on the F_2 generation as measured by the mutation rate.

When considering the effects of spermidine alone, the Kastenbaum-Bowman test demonstrated that spermidine had no significant mutagenic properties. In order to consider the validity of the data, the results were compared with another study in which a spermidine-only was run. The mutation frequency for the spermidine only treatment, 0.59%, is higher than Aschenbrener's (1972) value of 0.19%. Aschenbrener administered the spermidine and DMSO to the abdomen of the Drosophila with a topical injection using a syringe microburet. Aschenbrener also used a concentration of 0.02 M spermidine compared to the 100 mM used in this study, possibly explaining the higher number of sex-linked recessive lethal mutations found in the present study.

The control group, like the groups with which it was compared, had DMSO administered to it. This potent chemical might have affected mutagenesis. Kopeck (1973) obtained a 1.3% mutation rate with 100% DMSO topical administration, but Aschenbrener (1972) obtained a zero percent X-linked recessive lethal mutation rate for his DMSO only treatment. The value of 0.59% obtained in the present study for the DMSO controls, falls between the two values obtained by the other researchers. Using the Kastenbaum-Bowman test for comparing the values, the results of both researchers show no significant difference from the mutation rate at the 0.05 confidence level in the present study.

When considering the effect of DMSO on mutagenesis,

the spontaneous mutation rate must be reviewed. In the present study, historical controls were used. Lee et al. (1983) pointed out the advantage, primarily economical, of historical controls in their review of the sex-linked recessive lethal test. Their remarks were used as a basis for the decision not to run a control with only the Carpenter's media lacking brewer's yeast and agar. Wallace (1970) obtained a sex-linked lethal spontaneous mutation rate for a variety of wild-type males at 0.24% in 465 Drosophila. Kopeck (1973) reported a zero spontaneous mutation rate in 574 fertile F_1 matings. LeRoy (1972) and Aschenbrener (1972) reported mutation frequencies of 0.27% in 367 chromosomes and 0.26% in 372 chromosomes respectively. When comparing Kopeck's value for spontaneous mutation rate and the DMSO-only value from the present study, no significant difference is obtained. Due to the spontaneous control rates of the other researchers, the DMSO-only treatment did not significantly increase the number of sex-linked recessive lethal mutations from a nontreated control group. DMSO was shown to be slightly mutagenic in Kopecks' (1973), but he was using a 100% DMSO topical administration compared with the 1 M twenty-four hour feeding of DMSO used in the present study.

After evaluating the effects of DMSO on mutagenesis, the effects of the principle mutagen X-irradiation must be considered. The mutation frequency of 2.62% for a 2000 R

dose is lower than previous studies dealing with irradiation (Shiomi 1966; Herskowitz et al. 1959, cited by Herskowitz 1973). Shiomi obtained a value of 3.77% from 1301 chromosomes tested using a Canton-S stock for the tester strain. This value is not significantly higher than the mutation rate recorded in the present study for Oregon-R. Shiomi though had a lower mutation rate for 2000 R than Herskowitz's et al. (1959, cited by Herskowitz 1973) value of 5.80%. Two possible explanations should be considered for the differences in the mutation rate for the present study and the other studies. The first is that strains of Drosophila will differ in their response to mutagens and will also show different rates for spontaneous mutations. The second possible explanation may be that the 1 M DMSO used in the present study inhibited the mutation caused by X-irradiation. Mazar Barnett (1972) demonstrated that a 10% solution of DMSO would decrease the frequency of sex-linked recessive lethals by 1.06% when using a dose of 1000 R X-irradiation. The mechanisms proposed by Mazar Barnett included the DMSO possibly reversibly substituting for water in the hydration sheath of the nucleic acids, altering the configuration. This may cause the DNA structure to be less subject to radiation induced injury (Mazar Barnett 1972).

Based on Mazar Barnett's (1972) findings on the ability of DMSO to inhibit the mutation rate caused by

X-irradiation, another proposal may be given to explain the results of spermidine and X-irradiation on the mutation rate. Though no literature has been found at this time to support this, spermidine may act on the DNA structure in a manner similar to the DMSO. If the DMSO has the ability to undergo this reaction more quickly than spermidine, any further interaction of the DNA molecule with spermidine may be inhibited. Testing each chemical alone and using radioactive spermidine could allow one to determine if DMSO or spermidine reduces the X-irradiation induced mutagenesis.

One final point to be mentioned is the slight increase in mutation frequency of spermidine and X-ray treatment in comparison to the X-ray only treatment. Though the difference was statistically insignificant, it would be interesting to pursue investigations of the combined treatment by using a higher concentration of spermidine. The fact that spermidine binds to DNA is most obviously used to predict that it would decrease mutation rate, but that is unlikely in view of the present results. Possibly it enhances the mutation rate when combined with X-irradiation. Since X-irradiation may cause mutations via an indirect method, free radical formation, the spermidine may bind with the free radical. This may increase the radical's ability to interact with the DNA molecule and induce a mutation.

In conclusion, the present study has shown that a

specific concentration of spermidine administered via feeding had no effect in increasing or decreasing the induction of X-linked recessive lethal mutations in Drosophila by X-irradiation. Several recommendations for further study were mentioned to understand what the potential of spermidine may be for affecting mutagenesis.

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